بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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Detection of CYBA Gene Expression in Egyptian Children with Chronic Granulomatous Disease: A Pilot Study

Thesis

Submitted for the Partial Fulfillment of
Master Degree in **Clinical Pathology**

By

Rania Latif Shoukry
M.B., B. Ch. Faculty of Medicine, Ain Shams University

Under Supervision of

Prof. Nahla Mohamed Zakaria Yousef
Professor of Clinical Pathology
Faculty of Medicine, Ain Shams University

Prof. Abeer Al Sayed Ali Shehab
Professor of Clinical Pathology
Faculty of Medicine, Ain Shams University

Dr. Rasha Hassan El-Owaidy
Assistant Professor of Pediatrics
Faculty of Medicine, Ain Shams University

Dr. Marwa Rushdy El Najjar
Assistant Professor of Clinical Pathology
Faculty of Medicine, Ain Shams University

Faculty of Medicine,
Ain Shams University
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Rania Latif Shoukry

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List of Abbreviations

Abb.	Full term
A22	*Autosomal with mutations in p22phox gene CYBA.*
Allo-HSCT	*Allogenic Hematopoietic stem cell transplantation*
ALS	*Amyotrophic lateral sclerosis*
AML	*Acute myeloid leukemia*
AR	*Autosomal recessive*
AR-CGD	*Autosomal recessive CGD*
AUC	*Area under curve*
BCG	*Bacillus Calmette–Guerin*
cDNA	*Complementary deoxyribonuleic acid*
CGD	*Chronic granulomatous disease*
CHD	*Coronary heart disease*
CLL	*Chronic lymphocytic leukemia*
CRP	*C-reactive protein*
CYBA	*Cytochrome–bα.*
DHR	*Dihydrorhodamine*
DNA	*Deoxyribonuleic acid*
dNTP	*Deoxyribonucleotide triphosphate*
DUOX	*Dual oxidase*
dUTP	*Deoxyuridine 5-triphosphate.*
EL buffer	*Erythrocyte Lysis buffer*
ER	*Endoplasmic reticulum*
ESR	*Erythrocyte sedimentation rate*
ESRD	*End stage renal disease*
FACS analysis	*Fluorescence activated cell sorting*
FMLP	*Formylmethionyl-leucyl-phenylalanine*
G6PD	*Glucose-6-phosphate dehydrogenase*
GAPDH	*Glyceraldehyde 3-phosphate dehydrogenase*

List of Abbreviations **cont...**

Abb.	Full term
gDNA	*Genomic deoxyribonuleic acid*
gp91phox	*Glycoprotein 91 phagocytic oxidase*
GSH	*Glutathione*
GT-HSC	*Gene therapy for hematopoietic cells*
GTPase	*Guanosine triphosphate*
GVHD	*Graft-versus-host disease*
H2O2	*Hydrogen peroxide*
HIV	*Human immunodeficiency virus*
HLA	*Human Leukocyte Antigen*
HOCl	*Hypochlorous acid*
HSCT	*Hematopoietic stem cell transplantation*
IBD	*Inflammatory bowel disease*
IBM	*International Business Machines*
IFN-γ	*Interferon gamma*
IUIS	*International Union of Immunological Societies*
Kb	*Kilobase pair*
KCL	*Potassium chloride*
KDa	*Kilo Dalton*
LI	*Lytic index*
LPS	*Lipopolysaccharide*
MAC	*Myeloablative conditioning*
MM	*Multiple myeloma*
MPO	*Myeloperoxidase*
mRNA	*Messanger ribonucleic acid*
NADPH	*Nicotinamide adenine dinucleotide phosphate*
NBT	*Nitro-blue tetrazolium test*

List of Abbreviations **cont...**

Abb.	Full term
NCF	*Nuclear cytosolic factor*
NGS	*Next generation sequencing*
NOX	*Neutrophil oxidase*
NPV	*Negative predictive value*
OMIM	*Online Mendelian Inheritance in Man*
P22phox	*Protein22phagocytic oxidase*
PAGE	*Polyacrylamide gel electrophoresis*
PBS	*Phosphate buffered saline*
PCR	*Polymerase chain reaction*
PI	*Phagocytic index*
PID	*Primary immunodeficiency disorders*
PMA	*Phorbol myristate acetate*
PMNs	*Polymorphonuclear leukocytes*
PPV	*Positive predictive value*
Rac 2 gene	*Ras-related C3 botulinum toxin substrate 2*
RIC	*Reduced intensity conditioning*
RNA	*Ribonucleic acid*
ROI	*Reactive oxygen intermediates*
ROS	*Reactive oxygen species*
RT-PCR	*Reverse transcription polymerase chain reaction*
SOD	*Superoxide dismutase*
SPSS	*Statistical Package for the Social Sciences*
SYBR	*Synergy Brand*
TALENs	*Transcription activator-like effector nucleases*
XLR-CGD	*X-linked recessive CGD*
ZFNs	*Zinc-finger nucleases.*

INTRODUCTION

Immunodeficiency is the inability of body's immune system to perform its normal functions in protecting the host. Primary immunodeficiencies are hereditary, whereas secondary immunodeficiencies are acquired making them much more common ***(Chinen and Shearer, 2010).***

Primary immunodeficiency disorders (PIDs) are a group of genetic defects characterized by abnormalities of one or more components of the immune system. While there have been several advances in diagnosis, management and research in the field of PIDs, they continue to remain under-diagnosed, especially in the less affluent countries. More than 300 genetically defined single-gene inborn errors of immunity are being recognized as a cause of PIDs ***(Bousfiha et al., 2015).*** The increasing number of known types of PIDs in the past two decades is mainly due to the increasing knowledge of the function of immune system in addition to more accurate diagnostic methods ***(Bousfiha et al., 2013).*** Lack of awareness of PIDs is the major reason in delayed diagnosis and treatment resulting in morbidity and mortality ***(Nourijelyani et al., 2012).***

The International Union of Immunological Societies (IUIS) Expert Committee on Primary Immunodeficiency updated the classification of human primary immunodeficiencies into major groups of PIDs ***(Bousfiha et al., 2020):***

1- Immunodeficiencies affecting cellular and humoral immunity.
2- Combined immunodeficiencies with associated or syndromic features.
3- Predominantly antibody deficiencies.
4- Diseases of immune dysregulation.
5- Congenital defects of phagocyte number, function, or both.
6- Defects in Intrinsic and Innate Immunity.
7- Autoinflammatory disorders.
8- Complement deficiencies.
9- Bone marrow failure disorders.
10- Phenocopies of PID**.**

Chronic granulomatous disease (CGD), a rare primary immunodeficiency, is an innate, inherited, heterogeneous immunodeficiency disorder that renders patients susceptible to recurrent, severe pyogenic bacterial and/or fungal infections and excessive hyperinflammatory responses with eventual granuloma formation and premature death of the affected patients ***(Roos et al., 2014)***. This is caused by a defect in one of the subunits of the phagocyte-specific components of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase. These genetic defects lead to significantly decreased reactive oxygen species (ROS)

production, which plays a pivotal role in microbial killing. Mutations may affect one of the five genes (CYBB, CYBA, NCF1, NCF2, and NCF4) that encode structural components of the NADPH oxidase protein (gp91phox, p22phox, p47phox, p67phox, and p40phox, respectively). The gp91phox and p22phox proteins are located in the cell membrane and together form the flavocytochrome b558 of the NADPH oxidase. During phagocytosis, the cytosolic NADPH oxidase subunits p47phox, p67phox, and p40phox translocate to flavocytochrome b558 in the phagosomal membrane, inducing a conformational change that allows NADPH to donate electrons to gp91phox. This leads to the generation of superoxide in the phagosome, from which other ROS such as hydrogen peroxide may be formed ***(Fayyaz et al.,2020).***

Chronic granulomatous disease affects males more often than females. In North American and European studies, approximately two-thirds of individuals have the X-linked recessive form of the disorder ***(Marciano et al., 2018).*** Defects in gp91phox are an X-linked recessive (XLR-CGD) inheritance and are found in approximately 70% of CGD patients, who have the most severe phenotype. The autosomal recessive forms of CGD (AR-CGD) are due to mutations in the CYBA gene on chromosome 16 (5% of patients), the NCF1 gene on chromosome 7 (20%), the NCF2 gene on chromosome 1 (5%), or the NCF4 gene on chromosome 22 where only one patient was reported ***(Matute, 2009).***

Dihydrorhodamine test (DHR) is considered the gold standard for diagnosis of CGD using the mean fluorescence intensity of rhodamine 123 to quantitatively correlate it with reactive oxygen intermediate production and subsequent survival in patients with CGD. However, the oxidation of DHR also requires some myeloperoxidase activity and so its deficiency can appear with various levels of superoxide production on flow cytometry. Therefore, it is very important to determine the definitive molecular genetic defect using gene sequencing or other molecular techniques to diagnose ***(Yu et al., 2018).***